Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Sixty Years After "Isolating Mechanisms, Evolution and Temperature": Muller's Legacy

Norman A. Johnson

Department of Entomology and Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, Massachusetts 01003

THE major broad achievements by the luminaries of science often distract from our appreciation of their significant but narrower contributions to allied fields. One example is Linus Pauling's pioneering contributions to the molecular clock (Zuckerkandel and Pauling 1965); this work is far overshadowed by his monumental explorations of the nature of chemical bonds and the structures of proteins (Hager 1995). Another example, which is the focus of this article, is Herman Joseph (H. J., Joe to his friends) Muller's work in speciation genetics.

In this perspective, I discuss the legacy of the major ideas that emanate from Muller's 1942 article. My goal is to raise the profile of Muller's influence on speciation genetics for those more familiar with his contributions in other areas of genetics. As shown below, Muller played a major role in forming the conceptual framework of speciation genetics, including influence in topics such as evolution of hybrid incompatibilities, Haldane's rule, and the possibility of speciation without complete geographical isolation.

Muller's influence on diverse areas of genetics is truly impressive. He received the Nobel Prize for an extensive body of work on mutations and their induction by X rays (Muller 1927). Muller was also an important figure in the early Drosophila group and had a tremendous influence on Morgan and Sturtevant (Carlson 1981; Kohler 1994). Perhaps Muller's most important contributions are those he made to the fundamentals of genetics—including work on the size and nature of the gene, the gene as the basis of life, the relationship between gene and character, and the notion that mutable autocatalysis is the essence of life (Witkin 2001). In the functional interpretation of allelic series, Muller's "morphs" remain an active formalism to this day (Muller 1932). Within evolutionary genetics, Muller was the leading advocate for the "classical" position in the classical-balance school debate (Lewontin 1974). He is also known for his work on the evolution of sex, including the eponymous Muller's (1964) ratchet and for developing the concept of genetic load (Haldane 1937; Muller 1950; Crow 1993). Other essays in the *Perspectives* series that refer to Muller are Paul (1988), Lederberg (1991), Crow (1992), and Crow and Abrahamson (1997).

While Muller's contributions to speciation genetics are well known to researchers in that field, they are usually given at best brief mention elsewhere. In Carlson's detailed biography of Muller, the only mention of Muller's work in speciation is a relatively brief mention of his studies on the genetic basis of sterility in hybrids between *Drosophila melanogaster* and *D. simulans* (Carlson 1981, pp. 280–281). In actuality, Muller, like Theodosius Dobzhansky and Ernst Mayr, was one of the key architects in developing a genetic theory of speciation (Orr 1996).

Not even mentioned in Carlson's biography is a curiously titled article by Muller (1942), "Isolating mechanisms, evolution and temperature." This article grew out of a symposium address Muller gave at the American Society of Zoology meetings held in Dallas, Texas, at the end of December 1941. The title of his article reflects the name of the symposium in which it was given: Evolution and Temperature. Here, Muller presented a detailed review of what was known about speciation genetics at that time. Muller's article, however, is more than a review. He anticipated several developments in speciation genetics that would be rediscovered five or six decades later. Although neglected by Carlson (1981) and most other accounts of Muller's contributions, this article represents a foundation for research on the genetics of speciation. In this commentary I discuss the legacy of the major ideas that emanate from Muller's 1942 article.

GENETIC RECOMBINATION, ISOLATION, AND SPECIATION

Muller's article begins with the provocative first sentence: "Sexual reproduction is not an unmixed blessing". His point is that, while recombination provides

for favorable combinations of genes, it can also break down "combinations that were useful only in certain places or in connection with certain ways of living" (p. 71). This can decrease diversification and also limit adaptation.

Muller argued that diversification requires restriction of mixing between genetically different organisms. He noted that geographical barriers are one way to accomplish this. Geographical barriers are often temporary, and their elimination could result in the exchange of genetic variants between populations. Muller, like Dobzhansky (1937) and Mayr (1942, 1963), saw that permanent barriers to gene flow intrinsic to the organisms (isolating mechanisms) were needed for speciation and thus diversification. He also saw that isolating mechanisms expressed after zygote formation such as hybrid sterility and inviability are less sensitive to the environment and thus probably more permanent than prezygotic isolating mechanisms.

Muller was not wedded to a single mode of speciation. He noted that the reproductive isolation could arise from the cumulative effect of a multitude of different barriers to crossing. Unlike Mayr (1942, 1963), Muller did not think that absolute geographical barriers were required to initiate reproductive isolation and considered various ways that reproductive isolation could arise among organisms that were all in the same place (a.k.a., sympatric speciation). In particular, Muller anticipated that the antagonistic relationship between selection and recombination would be critical in models of sympatric speciation. As noted by Felsenstein (1981) and others (*e.g.*, Rice and Hostert 1993), selection for divergent phenotypes could promote sympatric speciation, but this process would be hindered by recombination.

The effects of genetic variants on traits (including fitness) often vary in different environments (Lynch and Walsh 1998). Wade (2000) has noted that the effects of this phenomenon, called genotype by environmental interaction (GEI), on adaptation will vary depending upon the extent of isolation, whether genetic or geographical. Without isolation, GEI can act as a constraint on local adaptation because the populations will be adapting to a global environment that may not fit a particular local environment. This constraint is lifted by allopatry or by genetic isolation. In fact, given isolation, GEI can promote local adaptation and diversification (Wade 2000).

THE DOBZHANSKY-MULLER MODEL AND EPISTASIS

What is the genetic basis of postzygotic reproductive isolation? How does it evolve? Although these questions remain foci of several contemporary research programs, Muller (1940, 1942) and the other founders of evolutionary genetics (*e.g.*, Dobzhansky 1937; Mayr 1942, 1963) had laid down the foundations for their answers more than a half century ago. In most contemporary models, hybrid fitness reduction is the consequence of deleterious interactions among genetic variants from the different nascent species and not simple gene action (reviewed in Johnson 2000; see below). These models are built upon a framework independently established by Dobzhansky (1937) and Muller (1940, 1942) and anticipated by Bateson (see Orr 1996). There is also substantial empirical evidence of interactions between different parts of chromosomes underlying hybrid fitness reduction (reviewed in Johnson 2000).

Bateson, Dobzhansky, and Muller all realized that the evolution of hybrid incompatibility would require more than a single genetic change. Muller (1942) succinctly explains the impossibility of hybrid incompatibility evolving via a single genetic change:

> Since practically all mutant genes must exist in heterozygous condition in the first individuals which inherit them, it is evident that any such lethal or sterilizing effect on the heterozygote would *ipso facto* incapacitate the very individuals necessary for the perpetuation of these genes. For this reason individual mutations causing complete hybrid incapacitation at one bound cannot become established (p. 84).

Muller then presented a series of simple verbal models for how hybrid incompatibilities could arise via two genetic changes. Here is one such model. Suppose that A and a are alleles at one locus and B and b are alleles at another locus. Further suppose that individuals with alleles a and b together are unfit. If an initial population of AABB individuals were split into two geographically isolated populations, hybrid incompatibility could evolve by having one population evolve to aaBB and the other to AAbb. F_1 hybrids would be AaBb and thus unfit. In such a scenario, maladaptive genotypes would not need to occur in the population ancestral to either of the descendant populations. Neither population need travel through an adaptive valley.

A consequence of these models, Muller (1942) realized, is that hybrid traits are not under natural selection. He noted:

> [T]he effects in question [hybrid dysfunction], detectable only on crossing, may legitimately be regarded as automatic by-products of the general differentiation produced by a combination of drift, and of selection for other characters than those here observed, utilizing gene mutations and to a much lesser extent, positional changes (p. 100).

In the terminology of evolutionary quantitative genetics, hybrid phenotypes can be considered as correlated responses to changes in the conspecific phenotypes (Johnson and Wade 1996; Johnson 2000). Unfortunately, we rarely know how evolutionary forces have acted upon conspecific phenotypes to generate the observed hybrid dysfunction. Indeed, we seldom know which conspecific phenotypes correlate with phenotypes of hybrid dysfunction.

Muller also realized that one consequence of these models is that all hybrid incompatibilities must be ini-

tially asymmetric. Recall the model above where combinations of the alleles a and b together are incompatible and the nascent species I and II are aaBB and AAbb, respectively. While a (species I) is incompatible with b (II), A (II) cannot be incompatible with B (I). Indeed, AABB is the ancestral genotype. It is possible that taxa I and II could further diverge at these loci to A1A1B1B1 and A2A2B2B2 where the A1-B2 and the A2-B1 combinations are both incompatible, but this scenario requires further evolution. The initial incompatibilities must be asymmetric.

Muller's intuition about the asymmetric nature of incompatibilities has been confirmed by subsequent formal modeling (*e.g.*, Orr 1995; but see Bordenstein and Drapeau 2001). Genetic mapping studies also strongly support this prediction. For instance, the hybrid sterility factors found when *D. simulans* is introgressed into *D. mauritiana* are not the same as those found in the reciprocal introgressions of *D. mauritiana* into *D. simulans* (*cf.*, Perez *et al.* 1993 and Palopoli and Wu 1994).

Building on the foundation set by Dobzhansky and Muller, Orr (1995) recently developed analytical models of the evolution of hybrid incompatibilities. From these models, Orr predicted a phenomenon called "snowballing". In this process, the number of genetic incompatibilities increases faster than linearly with genetic divergence, and at least as fast as the square of that divergence. Therefore, if genetic divergence increases roughly linearly with time, the number of incompatibilities would increase at least as fast as the square of the time separating the nascent species. Interestingly, Muller (1942) asserted that hybrid incompatibilities should increase more slowly than linearly (asymptotically) with divergence, but provided little justification. Neither snowballing nor a leveling off is apparent from the analyses of the patterns of speciation in both flies (Coyne and Orr 1997) and frogs (Sasa *et al.* 1998). These analyses show that hybrid sterility and inviability increase linearly with time. However, as Sasa *et al.* (1998) note, these studies measure only indices of hybrid sterility and inviability, and not the number of incompatibilities between the taxa. They also note that the indices are rather insensitive to the numbers of incompatibilities, since a single incompatibility is sufficient to generate hybrid malfunction.

Muller recognized that the models of hybrid fitness reduction that involve incompatible interactions arising from two genetic changes are only the simplest types of models and that incompatibility sets could involve more than two genes. He cited work by Gottschewski as an example of interaction of at least three genes in the sterility of hybrids between Drosophila species. During the 1990s, several examples of this phenomenon, named complex epistasis by Cabot *et al.* (1994) and Palopoli and Wu (1994), have been recorded in crosses between different species of Drosophila (reviewed in Wu and Palopoli 1994; Johnson 2000). Indeed, Wu and Palopoli (1994) question whether there is conclusive evidence that hybrid sterility in Drosophila crosses does not involve complex epistasis. One particularly striking example of complex epistasis occurs in the hybrid between *D. pseudoobscura* USA and *D. pseudoobscura* Bogota, where hybrid males are not sterile unless they possess the "right" allele at at least four loci (Orr and Irving 2001).

Cabot *et al.* (1994) and, more formally, Orr (1995) have examined models of complex epistasis. Their models suggest that reproductive isolation may evolve more easily when incompatibilities require three (or more) genes than when they involve only two. Given more loci in incompatibility sets, populations can transverse through more genotype space without encountering adaptive valleys. This increases the likelihood that populations will become incompatible with each other. Orr (1995) also showed that complex epistasis would actually accelerate the snowballing phenomenon—the number of incompatibilities between nascent species would increase at a rate even faster than the square of their genetic divergence.

With few exceptions, contemporary models of the evolution of hybrid incompatibilities are either variants or extensions of the Dobzhansky-Muller models. For instance, Sergei Gavrilets and his colleagues have developed "holey adaptive landscapes" models (Gavrilets 1997, 1999; Gavrilets *et al.* 2000). These models are essentially multilocus generalizations of the Dobzhansky-Muller models. In these models, individuals possess mating phenotypes that have additive genetic bases, and reproductive isolation occurs when the cumulative allelic difference between prospective mates crosses an arbitrary threshold.

Johnson and Porter (2000) presented a mechanistic model of how Dobzhansky-Muller incompatibilities could arise. They modeled the evolution of traits determined by interactions among genes in regulatory genetic pathways. Allopatric populations, subjected to directional but parallel selection, could respond to the selection pressure (changing optimal phenotype) in a variety of ways. Some of these responses are incompatible with others and would thus result in hybrids that had low or zero fitness.

Lynch and Force (2000) proposed a different mechanistic explanation for the acquisition of Dobzhansky-Muller incompatibilities—the fate of duplicated genes. Mutation and relaxed selection promote the degeneration of one copy of a duplicated gene. If in one population one copy of the gene degenerates and in the other population a different copy at a different chromosomal location loses function, some of the hybrids will have no functional copies. Lynch and Force (2000) also note that many genes are compartmentalized, having different sub-functions in the different domains. They show that this compartmentalization increases the likeli-

hood that the duplication and degeneration of genes will lead to fitness reduction in hybrids.

HALDANE'S RULE AND THE DOMINANCE THEORY

Eighty years ago, J. B. S. Haldane (1922) pointed out that whenever there is differential viability or fertility between the sexes in an interspecific cross, the more affected sex is usually heterogametic, the sex with heteromorphic sex chromosomes. This observation, now known as Haldane's rule, is one of the best-supported empirical generalizations about the patterns of speciation. During the 1980s and 1990s, after a long lull, many researchers searched for and tested various explanations of the cause of Haldane's rule. As this subject has been reviewed several times and these reviews generally concur about the explanations for the pattern (Wu *et al.* 1996; Laurie 1997; Orr 1997), I focus my review on the aspect of Haldane's rule that relates directly to the ideas in Muller (1942).

Muller (1942) proposed a genetic explanation for Haldane's rule: the dominance relations of alleles that cause dysfunction in hybrids. Muller, following Dobzhansky (1936, 1937), had noted that the X chromosome often appeared to have a disproportionate effect on the sterility of different classes of backcrossed males. Muller previously had attempted to explain this "X effect" and Haldane's rule as the consequence of X-autosome imbalance (see also Orr 1993; Turelli and Orr 1995). In the 1942 review, he stated clearly that both phenomena arise because the alleles that cause dysfunction in hybrids are generally recessive. Haldane's rule and the "large X effect," he stated (Muller 1942), arise

> . . . not primarily because the harmful genes in question are so much more apt to be in the X chromosome (though that is true to some extent of sterility genes) but because they are so apt to be recessive, and being recessive, would produce detectable results in the first generation only when they happen to be in the X chromosome (p. 89).

There is general agreement that the dominance relations of hybrid incompatibility alleles (Wu *et al.* 1996; Laurie 1997; Orr 1997; Turelli and Orr 2000) explain at least partially Haldane's rule and the apparent large X effect. High-resolution mapping of factors that cause sterility in hybrids between *D. simulans* and *D. mauritiana* shows that the X chromosome and autosomes have comparable densities of hybrid sterility factors (Hollocher and Wu 1996), but the X may have a somewhat higher concentration (True *et al.* 1996).

Muller also realized the potential of theoretical treatments of this dominance explanation. He said:

> . . . . it is possible to estimate the approximate likelihood of finding cases to which Haldane's rule applies, in comparison with those in which both sexes are similarly afflicted, on different assumptions regarding the frequency of establishment of dominant *vs.* recessive mutations, and of potentially harmful *vs.* potentially favorable mutations (Muller 1942, p. 91).

Indeed, Allen Orr and Michael Turelli have formalized such a mathematical theory of Haldane's rule on the basis of Muller's principle of the dominance of alleles (Orr 1993; Turelli and Orr 1995, 2000; Orr and Turelli 2001). From various data sets, they conclude that the alleles that cause hybrid dysfunction in crossses between sibling species of Drosophila are almost completely recessive, with the mean degree of dominance of these alleles being approximately 0.1 (reviewed in Turelli and Orr 2000).

ISOLATING MECHANISMS AND TEMPERATURE

The renewed interest in the genetic basis of hybrid incompatibility has led to a similar revival of interest in the effects that environmental factors (in particular, temperature) have on the severity of hybrid incompatibility. From a modest but growing set of studies on these environmental effects, the picture that changes in temperature and other factors often substantially alter hybrid traits is emerging (Bordenstein and Drapeau 2001).

Maside *et al.* (1998) examined temperature effects on spermatogenetic and sperm motility phenotypes associated with lines that had chromosomal regions of *D. mauritiana* introgressed into *D. simulans*. Males with these introgressed segments are usually fertile, but spermatogenesis is substantially delayed, particularly at lower temperatures. Such studies suggest that the outcome of genetic studies of hybrid sterility and inviability may be influenced by experimental conditions including temperature.

F_1 crosses between the flour beetle species *Tribolium castaneum* and *T. freemani* are usually female biased, apparently owing to male mortality. Moreover, a substantial proportion of the surviving hybrid males, but not the hybrid females, have antennal and leg deformities when reared under standard laboratory conditions at 29° (Wade and Johnson 1994; Wade *et al.* 1999). These traits—male rarity and male deformity—are consistent with Haldane's rule because males are the heterogametic sex in this genus. Substantial genetic variation exists both within and among wild-caught populations and laboratory strains (Wade and Johnson 1994; Wade *et al.* 1997, 1999). Wade *et al.* (1999) studied the effects of temperature on the proportion of males in F_1 crosses between the flour beetles *T. castaneum* and *T. freemani* and the frequency and severity of deformities in surviving male hybrids. At higher temperatures, the mean expression of these aspects of Haldane's rule increased. Furthermore, female hybrids of some of the crosses had some deformities when reared at the higher temperature. There was also substantial GEI for expression of these traits.

Bordenstein and Drapeau (2001) have modeled the effects of environmental factors and GEI for hybrid fitness. They reached three conclusions. First, they find that relaxing the assumption of no environmental effects on hybrid fitness allows for the possibility of hybrid incompatibility via environment-dependent, single-locus underdominance (*i.e.*, the heterozygote is less fit than either homozygote). This is in stark contrast to traditional models, which find hybrid fitness reduction exceedingly unlikely to evolve with only a single locus. Second, the incorporation of GEI into traditional Dobzhansky-Muller models increases the number of potentially negative epistatic interactions causing hybrid incompatibilities. Third, GEI can also affect many of the predictions made by Orr (1995) that arise from Dobzhansky-Muller models. For instance, incompatibilities need not be initially asymmetric in the GEI model as they are in the traditional models (Bordenstein and Drapeau 2001).

MULLER AND THE ECLIPSE OF SPECIATION GENETICS

From the early 1940s through the early 1980s, little research was done in speciation genetics. Dobzhansky's (1937) *Genetics and the Origin of Species* devoted several chapters to speciation, but soon after its publication, Dobzhansky's contributions to speciation rapidly dwindled. After this 1942 review, Muller did not continue work on speciation. Howard *et al.* (2002) speculated that Dobzhansky's interest in speciation waned quickly as he became involved in the *D. pseudoobscura* fieldwork necessary to study the genetics of natural populations. If Dobzhansky was too busy to continue work on speciation genetics, Muller was even more so. His career at the time was extremely unsettled (Carlson 1981). After returning from Europe, Muller spent the wartime years at Amherst College in a nontenured teaching position with scant laboratory facilities. With a young child from his second marriage, Muller also had other demands on his time and energy (Carlson 1981). In addition to these time constraints, it is plausible that Muller and Dobzhansky, along with their contemporaries and immediate descendants, considered the genetics of species formation, especially allopatric, essentially solved. They had established an essential framework for how, given geographical isolation, reproductive isolation could evolve via the same forces that govern evolution within species. Sympatric and parapatric speciations were less clear. Perhaps, had Muller and Dobzhansky continued to explore these models, we might today be much further along in our understanding of the genetic processes that govern the origin of species.

I thank Seth Bordenstein, Michael Wade, and Mohamed Noor for many helpful comments. I am also grateful for support from the National Science Foundation (DEB 0075451).

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